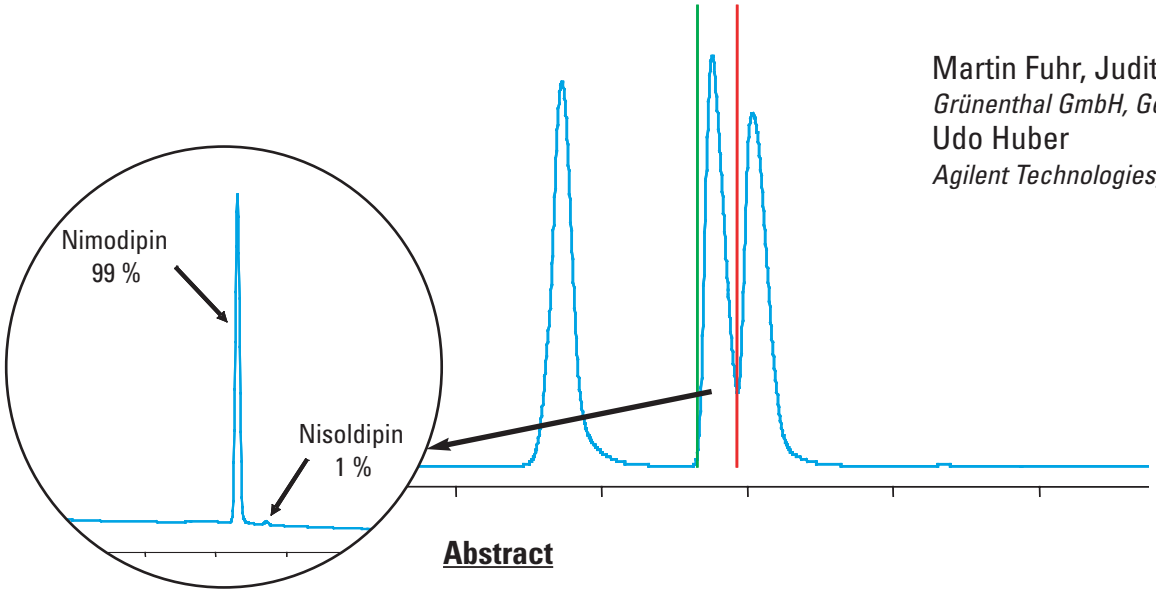
Configuring a mass-based fraction collection system for highest purity

Application Note



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Abstract

Preparative HPLC with mass-based fraction collection is the purification method of choice in modern, high-throughput drug discovery. Since the MSD is a destructive detector, a flow splitter is required in the system to split a small part of the flow into the MSD for detection of the target mass and triggering of the fraction collector. Most commercially available flow splitters achieve the split with one or two tees and capillaries of different length and diameter. The Agilent active splitter, however, achieves the split using a rapidly switching valve system. The splitter design and the location of the splitter in the configuration have a significant influence on the purity of collected fractions, which is the most important parameter for subsequent biological testing. In this Application Note different splitter designs and configurations of the splitter in the purification system are compared in relation to the purity of collected fractions.

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Introduction

Purity, recovery and throughput are the most important parameters for any preparative HPLC application. Since they are dependent on each other, it is not possible to optimize a method relating to all three parameters. The application determines the parameter for which the method is optimized. Recovery is probably the most important parameter for the purification of reaction intermediates and purity is the most important for compounds that are submitted to biological screening. Since the molecular mass of these compounds is known by the chemists, preparative HPLC with mass-based fraction collection is usually the purification method of choice. An important part of a mass-based fraction collection system is the flow splitter[2] which also has a tremendous influence on the purity of the collected fractions. The influence of the splitter design and the correct configuration of the flow splitter in the system are discussed in this application note.

Equipment

The experiments were performed on an Agilent 1200 Series purification system containing the following modules:

- 2 Agilent 1200 Series preparative pumps
- Agilent 1200 Series dual-loop autosampler PS (1000-µL loop)
- Agilent 1200 Series column organizer
- Agilent 1200 Series multiple wavelength detector (flow cells: 0.06 and 10-mm path length)
- Agilent 1200 Series fraction collector PS
- Agilent 1200 Series MSD
- Agilent 1200 Series isocratic pump (make-up pump)
- Agilent Active splitter or commercially available passive flow splitter (1:1000)
- Agilent 1200 Series UIB

The system was controlled using the Agilent ChemStation (rev. B.02.01).

Results and discussion

Splitter design

The most commercially available flow splitters are passive splitters (figure 1a). The split is achieved by using tubing of various lengths and diameters, resulting in different back-pressures. The Agilent active splitter (figure 1b) has an unique working principle: a rapidly switching valve transfers a certain volume of mobile phase actively from the main flow into the make-up flow. The split ratio is determined by the switching frequency of the valve. The splitter design also has an influence on the purity of the collected fractions. The long tubing required to achieve the split in the passive splitter leads to a significant peak broadening and therefore to a partial remixing of peaks that were separated on the column, for example. Furthermore the system configuration leading to the best

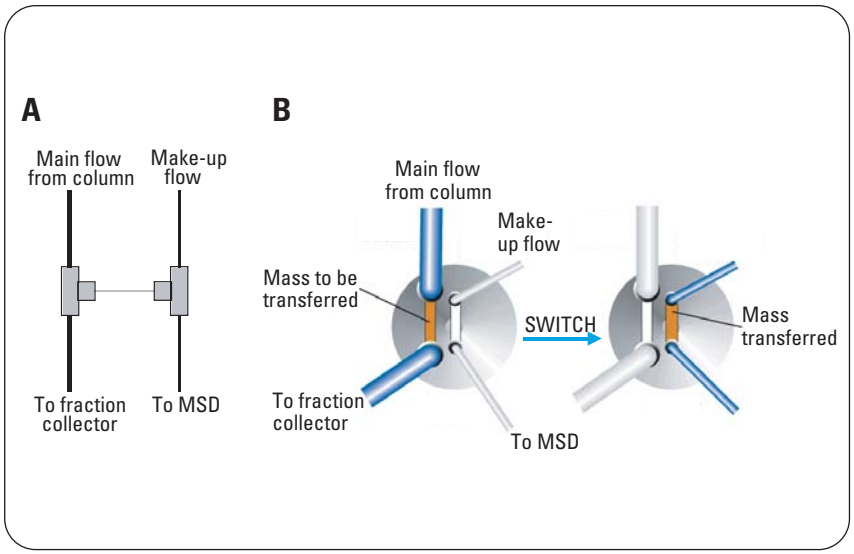

Figure 1
A) Passive splitter B) Active splitter.

purification results is not possible with the passive splitter because its high back-pressure could damage the flow cell of the UV detector. The advantages and disadvantages of each splitter design are summarized in table 1.

Passive splitter	Active splitter
High back-pressure	Almost no back-pressure
High delay volume leads to peak dispersion and, therefore, lower purity of fractions	No delay volume, no peak-dispersion
Fixed split ratio	Adjustable split ratio
Split ratio depends on viscosity of mobile phase of main flow and make-up flow, changes across gradient	Split ratio not influenced by mobile phase composition
Change of split ratio (i.e. exchange of splitter) changes delay time/volume	Change of split ratio (switching frequency) does not influence delay time/volume
Almost impossible to repair if clogged	No clogging, if clogging occurs rotor seal and stator face assembly can easily be replaced
No maintenance necessary	Replacement of rotor seal and stator face assembly necessary

Table 1
Advantages and disadvantages of splitter designs.

Sample and chromatographic method

The sample used for the experiments contained the compounds nifedipin, nimodipin and nisoldipin (15 mg each in 500 µL of DMSO). Nimodipin and nisoldipin are not base line separated, as shown in figure 2, using the selected set-up method. The purity of the fraction containing nimodipin was measured to identify the optimal configuration. The purity was determined by analytical HPLC with UV detection. The purity of nimodipin in the fractions was determined based on area percent despite the fact that the compounds do not have the same absorption coefficients. For each configuration fractions were collected based on the UV signal (slope only, up- and down slope 10 mAU/s), based on the MS signal (threshold only, 200000 counts) and using the AND combination of the UV and MS signal (UV: slope only, up and down slope 10 mAU/s, MS: threshold, 200000 counts). Each experiment was repeated three times and median results were taken.

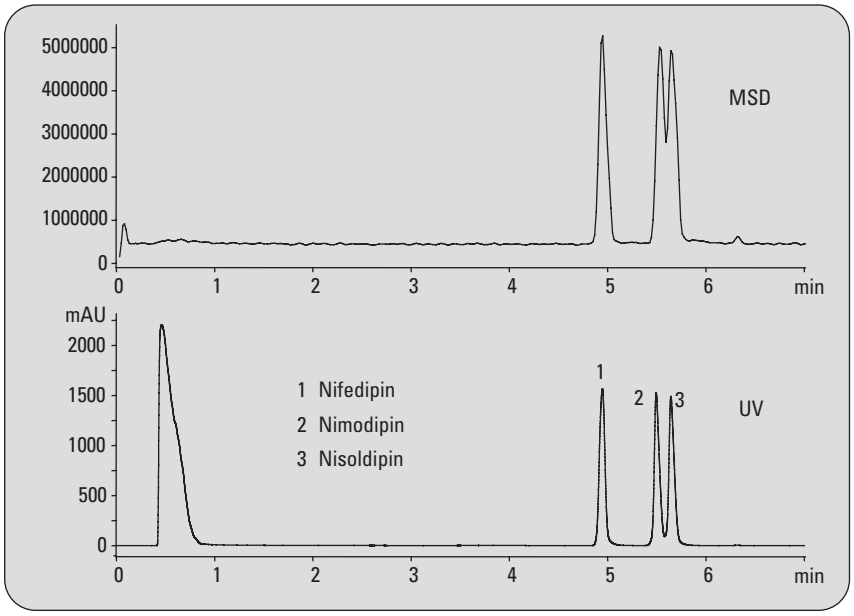

Figure 2
Sample for purity measurements.

Chromatographic conditions

Column:	ZORBAX SB- C18, 21.2 x 50 mm, 5 µm
Mobile phases:	Water = A, Acetonitrile = B
Gradient:	at 0 min 10 % B at 2 min 10 % B at 6 min 90 % B at 7 min 90 % B
Stop time:	7 min
Post time:	3 min
Flow:	25 mL/min
Injection volume:	500 µL
Column temperature:	ambient
UV detector:	DAD 220 nm/4 (ref. off) flow cell 10 mm pathlength
Polarity:	positive
Mode:	scan (200 – 700)
Make-up solv.:	Water/methanol 20:80 + 0.1 % HCOOH
Make-up flow:	1 mL/min
Split ratio:	1:9500 (active splitter) 1:1000 (passive splitter)

Configuration 1:
UV detector in front of splitter

This configuration (figure 3) is only possible with the active splitter because the passive splitter creates a back pressure too high for the flow cell of the UV detector. The purity results for UV, MS and combined fraction collection are shown in table 2. As shown in a previous Application Note[3] UV-based fraction collection results in the highest purity, whereas the purity using mass-based fraction collection is about 7 % lower. This is due to the broader peaks in the MSD.

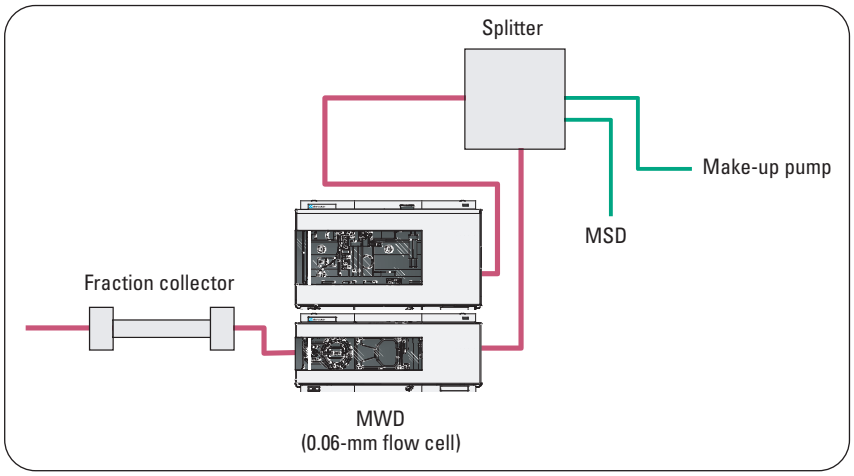

Figure 3
Configuration 1: UV detector in front of splitter.

Splitter	Collection	Purity nimodipin
Active	UV	99.4 %
	MS	92.1 %
	UV AND MS	98.8 %

Table 2
Purity results configuration 1.

Configuration 2:
UV detector between splitter and MSD

Experiments using configuration 2 (figure 4) were done with the active and passive splitters. The results are shown in table 3. As shown in table 3, the purity results achieved with the passive splitter are always slightly lower than with the active splitter. Furthermore purities are lower than with configuration 1, especially when using mass-based fraction collection. The reason for this is that the flow cell of the UV detector adds additional peak broadening to the MSD signal.

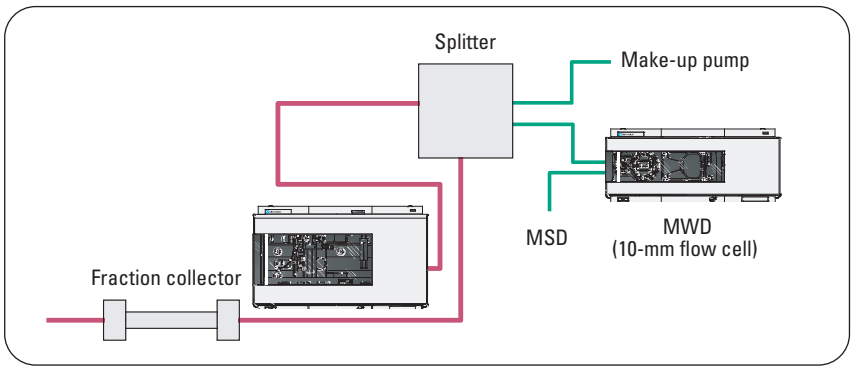

Figure 4
Configuration 2: UV detector between splitter and MSD.

Splitter	Collection	Purity nimodipin
Active	UV	98.4 %
	MS	85.4 %
	UV and MS	94.5 %
Passive	UV	97.3 %
	MS	81.0 %
	UV and MS	93.0 %

Table 3
Purity results configuration 2.

Configuration 3:
Second splitter to UV and MS detector

In this configuration the flow coming from the active or the passive splitter was split again to the UV and to the MS detector using a tee. The split ratio achieved by the back pressures of the detectors was not determined. Experiments using configuration 3 (figure 5) were done with the active and with the passive splitter. The results are shown in table 4. Using a second splitter removes the UV flow cell from the flow path going to the MSD, therefore less peak-broadening and higher purity is observed, especially for mass-based fraction collection. Overall the purity results are higher than for configuration 2, but not as good as for configuration 1. Again, the results for the active splitter are better than for the passive splitter.

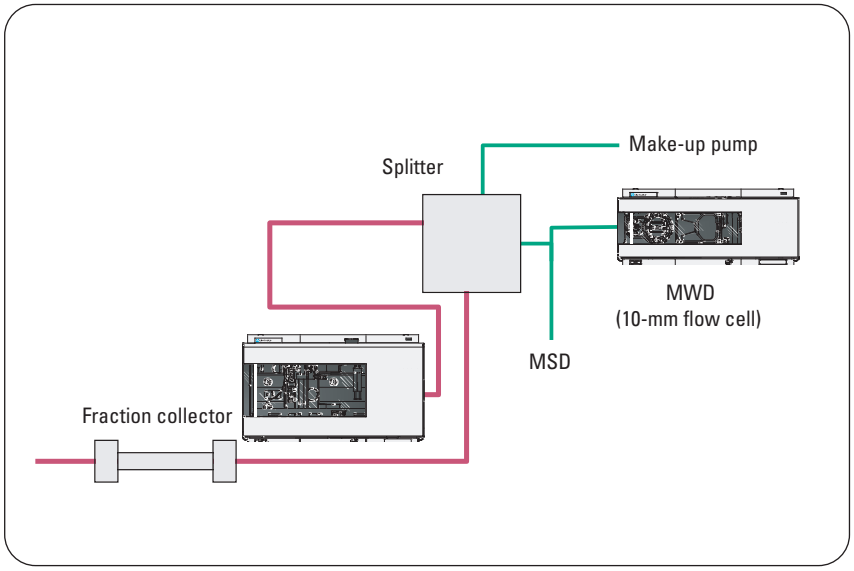

Figure 5
Configuration 3: Second splitter to UV and MS detector.

Splitter	Collection	Purity nimodipin
Active	UV	98.3 %
	MS	89.2 %
	UV and MS	95.2 %
Passive	UV	98.2 %
	MS	83.1 %
	UV and MS	94.2 %

Table 4
Purity results configuration 3.

Conclusions

As already published in a previous Application Note, the purity results for UV based fraction collection are always better than for mass-based fraction collection. To re-gain the advantage of the high selectivity of the MSD the UV and MS can be combined using a logical AND combination; the purity results for this combination are almost as good as UV based fraction collection.

The configuration which provides the best purity results, especially for mass-based fraction collection, places the splitter directly after the UV detector. In this configuration neither the UV detector flow cell nor an additional tee splitter leads to further peak broadening of the MS signal. However, this configuration can only be set up using the active splitter because the back pressure introduced with the passive splitter is too high for the UV flow cell. If, for any reason, the UV detector has to be placed after the splitter, for example, if only an analytical flow cell is available or in a combined analytical and preparative system[3], the introduction of a second splitter for parallel flows to UV and MS detector is advantageous. The results of the purity experiments are summarized in figure 6.

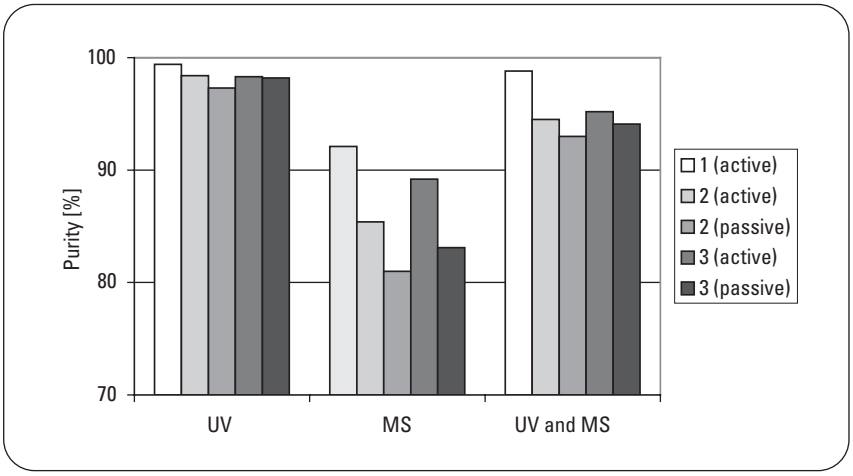

Figure 6
Results of purification experiments.

Summary of the results from figure 6:

- Purity results are best for UV based fraction collection and unsatisfactory for mass-based fraction collection. The AND connection of UV and MS combines the high purity of UV based fraction collection with the selectivity of the MSD.
- Using the same configuration purity results achieved with the active splitter are always higher than with the passive splitter.
- The optimum configuration places the UV detector between the column and the splitter. This is only possible with the active splitter.
- If the UV detector must be placed after the splitter, a configuration with a second splitter leads to better purity results. The second splitter can be a simple tee.

References

1.
Experiments based on a similar topic and in answer to the same questions have been simultaneously performed by Judith Steffens, Grünenthal GmbH, Aachen, Germany. The results will be published in her diploma thesis entitled “Hochdurchsatzaufreinigung von 3-Amino-Imidazol [1,2-a] pyridinen und -pyrimidinen mit verschiedenen Split-Techniken und konfigurierten HPLC-MS Systemen”, **2006.**

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